Blood Group Genotyping in Germany

FDA, Workshop on Molecular Methods in Immunohematology
Lister Hill Auditorium, NIH Bldg 38A
Bethesda 25 – 26 Sept 2006

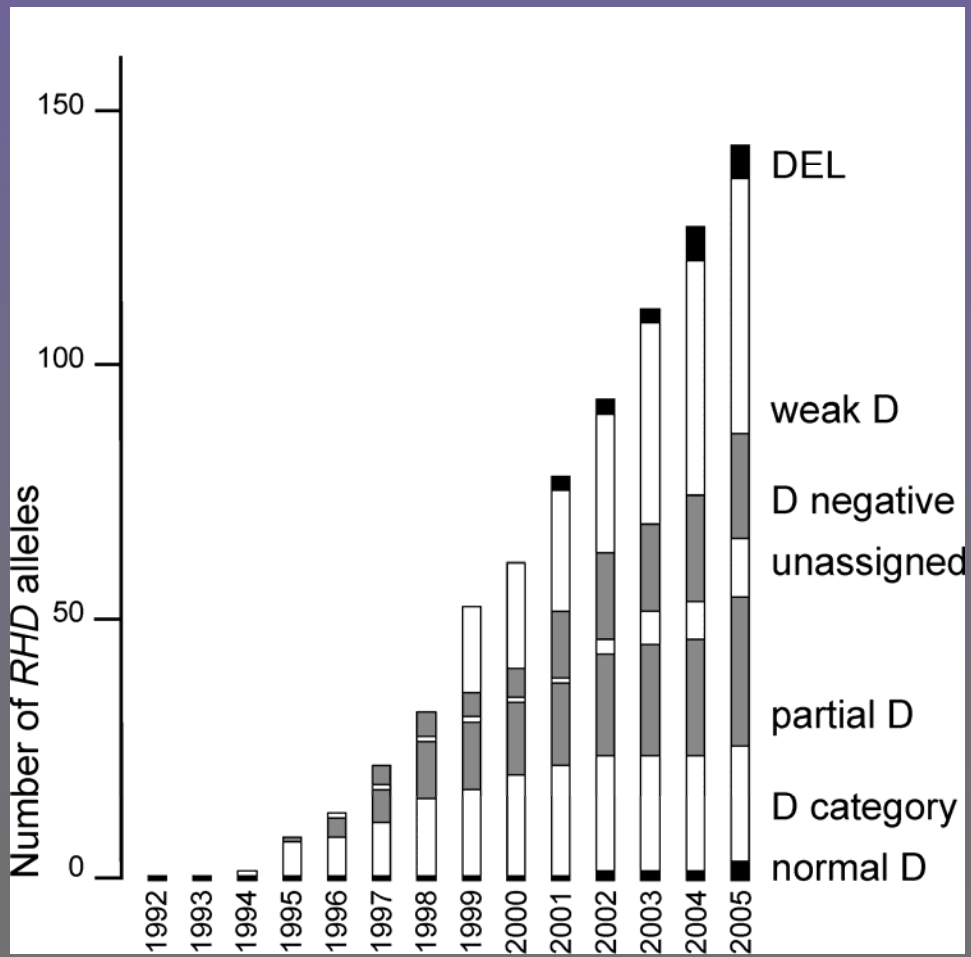
Willy A. Flegel MD
Professor of Transfusion Medicine

Institut Transfusionsmedizin, Universitätsklinikum Ulm
Institut für Klinische Transfusionsmedizin und Immungenetik Ulm gGmbH (IKT Ulm)

Clinical management of D antigen in Germany

- two anti-D monoclonals
  - that do not bind DVI
  - German guidelines since 1996
- RhIg
  - prenatal & post partum
- CcEe antigens, if
  - girl
  - woman < 45 years
  - multiple transfusions
  - immunhematologic problems

- D antigen most immunogenic
  - because RhD protein is completely lacking in D neg.
- most important blood group system encoded by proteins



- Transfus Clin Biol 13(2006)4

Clinical applications

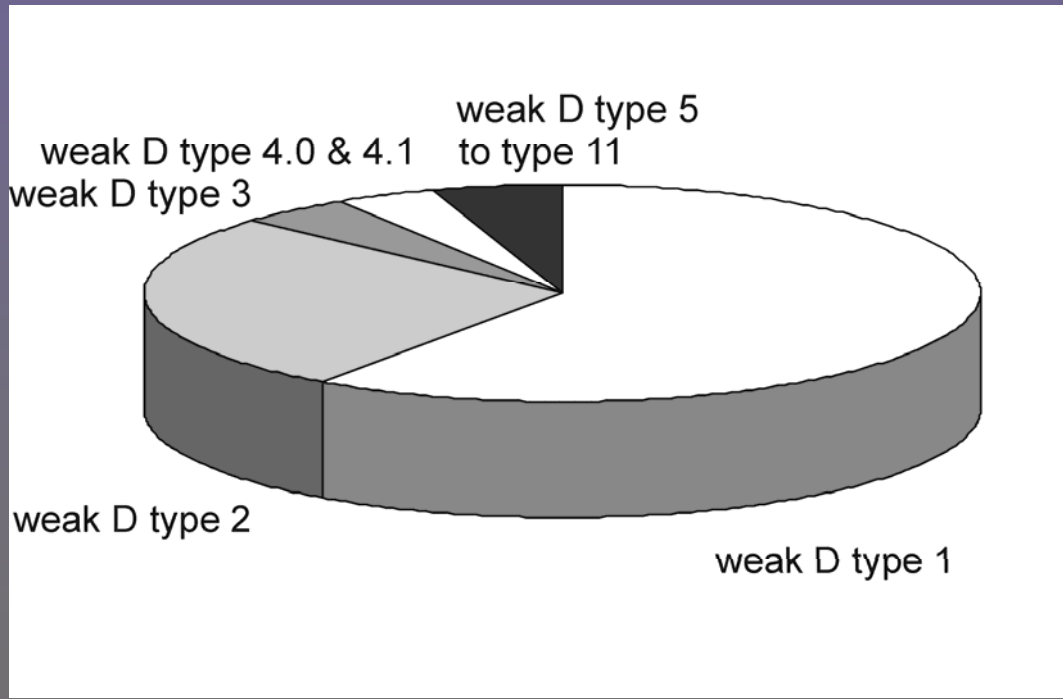
- Patients with weak D phenotypes
  - prevalent weak D should be transfused D pos.
- Maternal care
- Blood donors
- Anti D and family planning

Distribution of molecular *weak D types* in Europeans

- weak D type 1 to 4: 94%
- less frequent weak D types combined: 6%

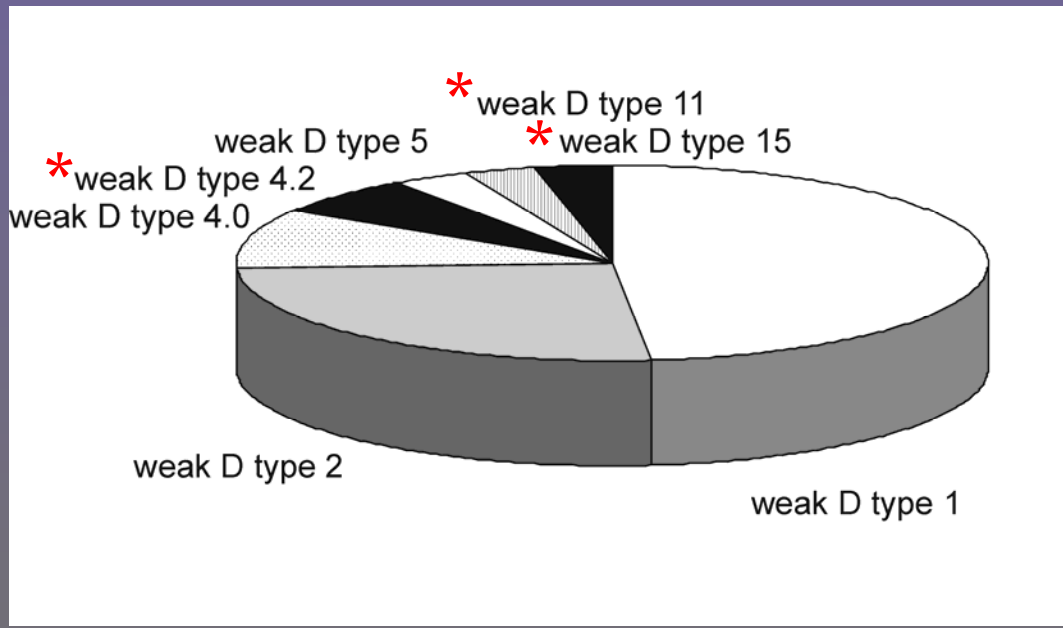
- often confirmed in Europeans
- details vary among European populations
- e.g. Portuguese: weak D type 2 most frequent

  - Blood 93(1999)385



weak D types among transfusion recipients with anti-D

- 31 observations since 1998
- prevalent weak D: auto-anti-D only n=24
- weak D type 4.0 n=3, low titer
- allo-anti-D * n=4
  weak D type 4.2, 11 & 15
  - Blood 95(2000)2699 http://www.uni-ulm.de/~wflegel/RH/RIR/#title



Clinical management of patients with a weak D phenotype

Table 1 ***Weak D types*** **that are prevalent or clinically relevant in Europeans**

Weak D phenotype	Prevalence in Germany	Haplotype association	Transfusion recipients with anti-D (*n*)*			Recommended management		Clinical consequences
			Total	Allo- anti-D	Auto- anti-D	RBC unit transfusion	RhIg in pregnancy?	
Type 1	0.2964%	*CDe*	15	0	15	D-pos.	No	Established
Type 2	0.0759%	*cDE*	8	0	8	D-pos.	No	Established
Type 3	0.0219%	*CDe*	0	n.a.	n.a.	D-pos.	No	Established
Type 4.0	0.014%	*cDe*	3	See remark	See remark	D-pos.	No	Pending
Type 4.1	0.023%	*cDe*	0	n.a.	n.a.	D-pos.	No	Established
Type 4.2	Rare	*cDe*	2	1	1	D-neg.	Yes	Established
Type 5	0.0035%	*cDE*	1	0	1	D-neg.	Yes	Pending
Type 11	>0.0009%, 0.0009%	*CDe, cDe*	1	1	0	D-neg.	Yes	Established
Type 15	Rare	*cDE*	1	1	0	D-neg.	Yes	Established
Type 19	0.067%	*CDe*	0	n.a.	n.a.	D-neg.	Yes	Pending
Type 20	0.024%	*cDE*	0	n.a.	n.a.	D-neg.	Yes	Pending
Other types	Rare	Variable	0	n.a.	n.a.	D-neg.	Yes	Pending

– Flegel WA: Curr Opin Hematol 2006 in press

Patient testing for clinically relevant weak D types

- may save 3% – 5% of all D neg. units for D neg. recipients who really need them
- one test per patient
- CE-labelled test kits
- offered as a service in Germany since 2000

  - protocol details published in e.g.
    - Flegel WA
      Curr Opin Hematol 2006 in press

Clinical applications

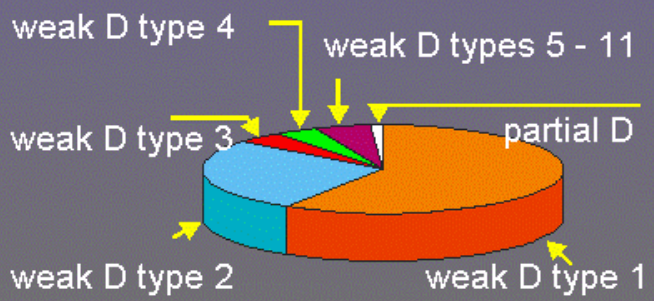
- Patients with weak D phenotypes
- Maternal care
  - prenatal diagnostics
  - RhIg in pregnancy
- Blood donors
- Anti D and family planning

Prenatal diagnostics

- blood group genotyping is standard care
  - puncture of fetal cord contra-indicated, if done for blood group typing only
    - German consensus statement Infusionsther Transfusionsmed 27(2000)215
- typical diagnostic requests in Germany
  - from amniotic fluid or its cell culture
    - in order of frequency:
  - D
  - Rhesus phenotype (CcEe)
  - K
  - Fy, Jk etc. (sporadic requests only)
- fetal *RHD* from maternal plasma
  - not widely available in Germany at this time

RhIg during pregnancy may be dropped, ...

- ... if mother carries certain weak D types
- ... if fetus types D neg. from maternal plasma



- Blood 93(1999)385
- Curr Opin Hematol 2006 in press

Noninvasive Prenatal Diagnosis of Fetal Rhesus D

Ready for Prime(r) Time

Diana W. Bianchi, MD, Neil D. Avent, PhD, Jean-Marc Costa, PhD, and C. Ellen van der Schoot, MD, PhD

- Obstet Gynecol 106(2005)841

Health and cost benefits RhIg may be dropped, ...

- ... if mother carries certain weak D types

- may save 3% – 5% of all anti-D shots
- one test per mother‘s lifetime
- test pays for itself
  - health benefit for mother may come for free

- ... if fetus types D neg. from maternal plasma

- may save 40% of prenatal anti-D shots
- one or more tests per pregnancy
- test may pay for itself

Regulatory issues
RhIg may be dropped, ...

- ... if mother carries certain weak D types

- CE-labelled test kits
- compatible with current guidelines
- offered as a service in Germany since 2001

- ... if fetus types D neg. from maternal plasma

- no test kit available
- guidelines would need to be relaxed
- currently not offered as a service in Germany

Clinical applications

- Patients with weak D phenotypes
- Maternal care
- Blood donors
  - screen for DEL, weak D and D^+/D^- chimera among serologically D neg. donors
- Anti D and family planning

Rationale: to improve RBC unit safety

- potential immunogenicity of weak D, DEL and D^{+}/D^{-} chimera
- *RHD* with very weak D antigen expression
  - detectable by adsorption/elution or flow cytometry
    - it should be remembered: there are *RHD* alleles without any serologically detectable D antigen
    - Transfusion 45(2005)1547

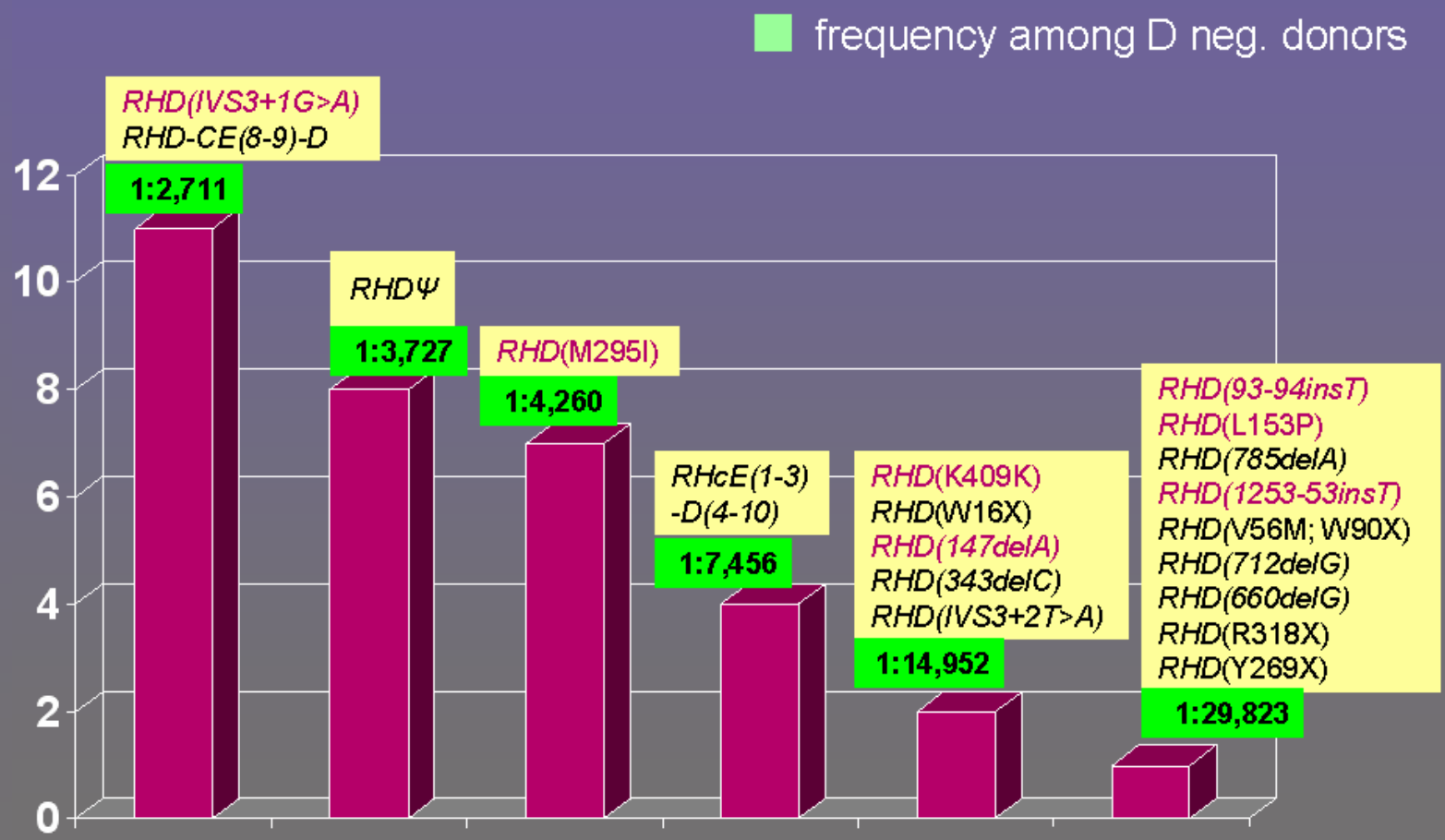
Routine testing 1/02 – 12/05 at our blood service

- testing of all first time donors for D antigen
  - serology according to German guidelines
  - oligoclonal anti D plus antiglobulin test in gel technique
- screening of all serologically D negative first time donors for *RHD* gene
  - 29,823 in 4 years
- PCR screening of pools of 20 donors
  - PCR-SSP for *RHD* intron 4
- all novel *RHD* alleles characterized
  - PCR, sequencing, testing for DEL

Distribution among Rhesus phenotypes

Phenotype	N	*RHD* positive	DEL positive
Ccddee	27,859	8	0
Ccddee	1,241	44	23
ccddEe	679	7	1
CCddee	20	2	2
CcddEe	19	0	0
ccddEE	4	0	0
CCddEe	1	0	0
total	29,823	61	26

Frequency distribution



RHD alleles expressing DEL differ among ethnic groups

Population	Prevalence in D-negative blood donors: Any *RHD* allele	Prevalence in D-negative blood donors: DEL phenotype	Prevalent *RHD* alleles
Europeans	0.2%	1 in 1000	*RHD*(IVS3+1G>A), *RHD-CE(8-9)-D*, *RHDΨ*, *weak D type 11* in CDe*
Africans	10%	<1 in 100	*RHDΨ*, *Cde*^{s}
East Asians	30%	1 in 3	*RHD*(K409K), *weak D type 15*, *weak D type 17*

– Flegel WA: Curr Opin Hematol 2006 in press

RHD genotyping in donors: improves RBC unit safety

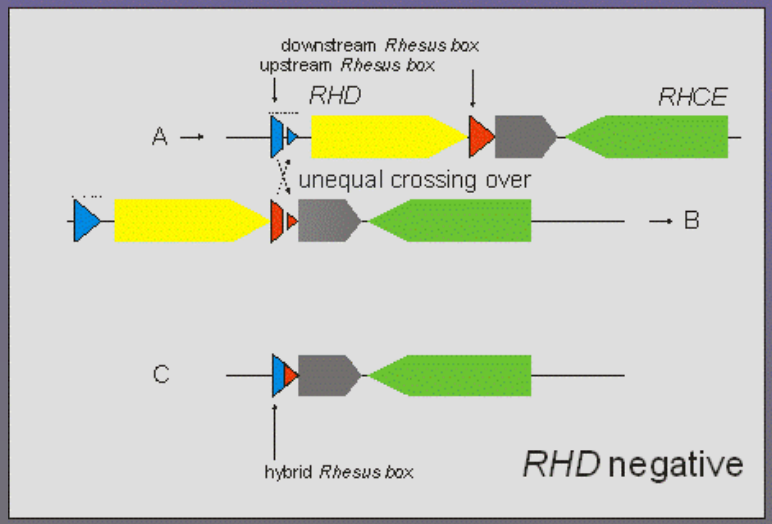
- by moving donors with
  - weak D, DEL & D^+/D^- chimeric RBC populations
- to the D positive donor pool
- obviates need for very sensitive anti-D test and its quality assurance (cost savings)
- adaptation to allele distributions in different populations required
  - protocols for e.g. East Asia Transfusion 45(2005)345 and in press

Clinical applications

- Patients with weak D phenotypes
- Maternal care
- Blood donors
- Anti D and family planning
  - genotype father for *RHD* heterozygosity, if mother carries anti D

Detection of *RHD* heterozygous status in fathers

- hybrid *Rhesus box*



- Problem
  - *Rhesus box* variants
- Blood 95(2000)3665

- *RHD/RHCE* dosage

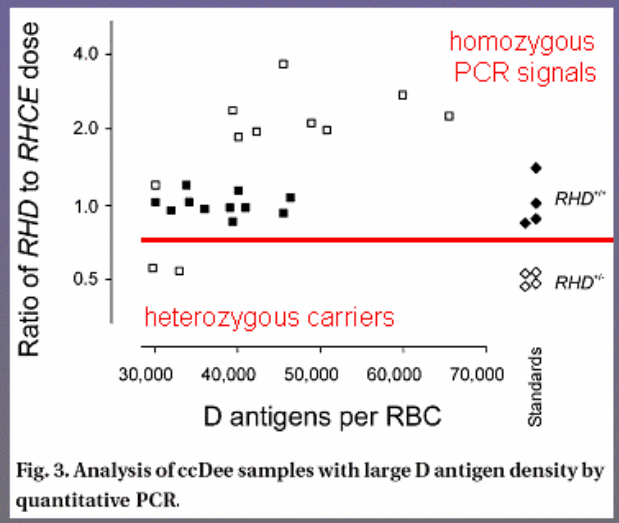
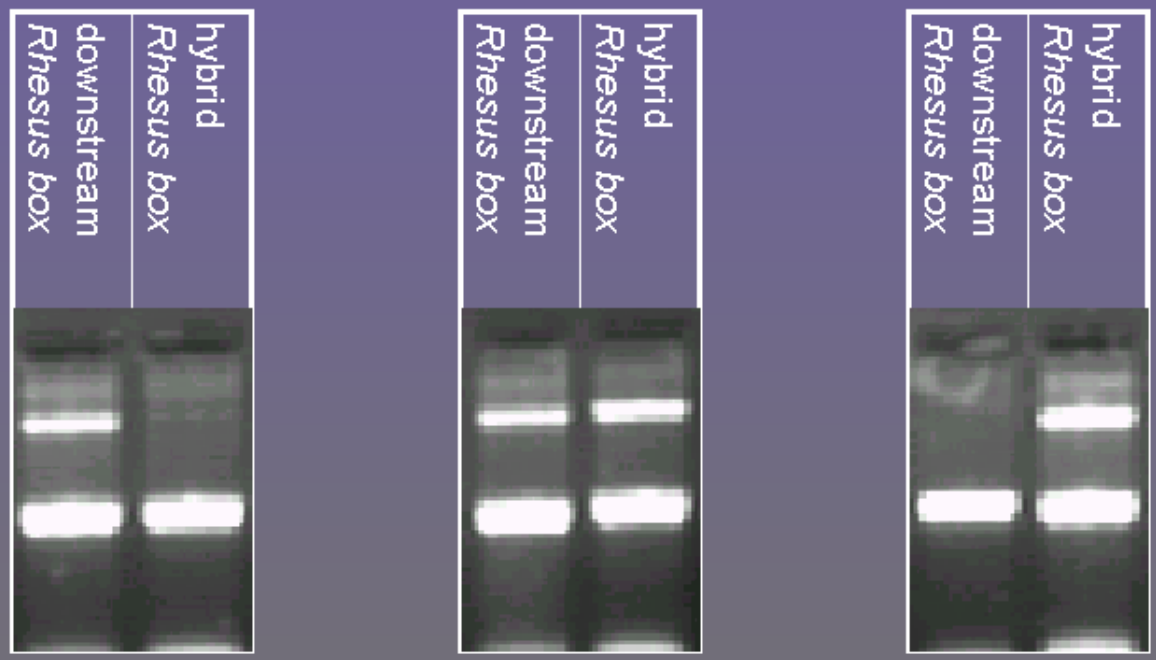


Fig. 3. Analysis of ccDee samples with large D antigen density by quantitative PCR.

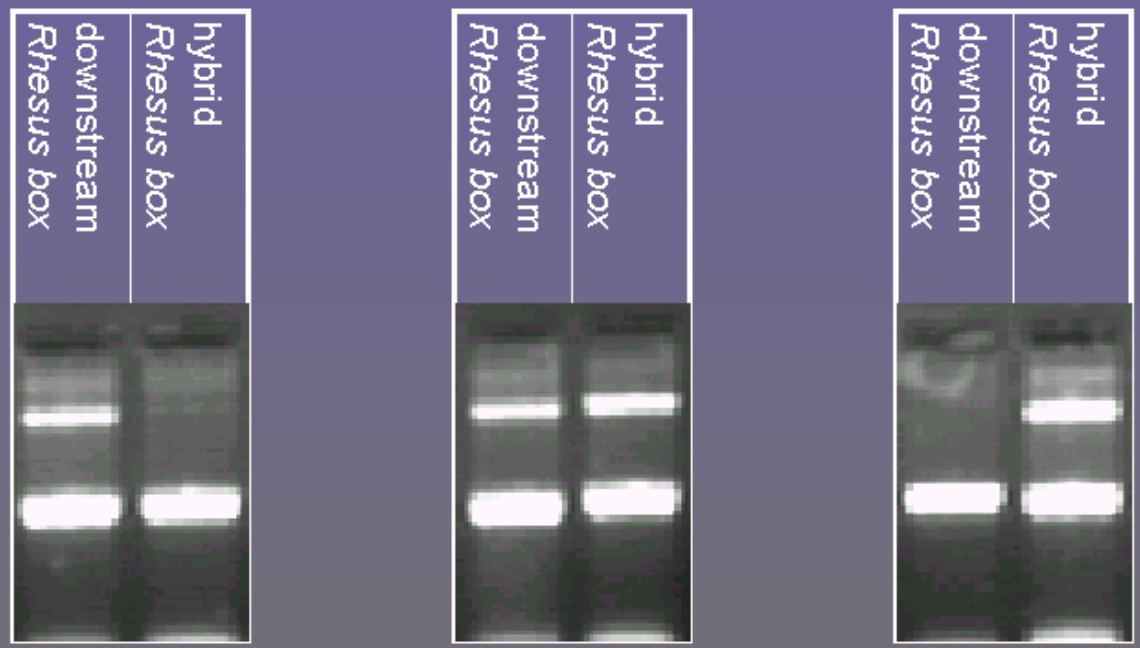
- Problem
  - *RHCE-D-CE* alleles, like *D--*
- Transfusion 46(2006)1343

PCR-SSP for *RHD* deletion



- detection of *RHD* heterozygosity in a father

RHD genotyping



DD

homozygous

Dd

heterozygous

dd

Regulatory and liability issues *RHD* heterozygous status

- hybrid *Rhesus box*
- PCR with sequence specific primers (PCR-SSP)
- Problem
  - *Rhesus box* variants primarily in Africans
- Resolution
  - *RHD/RHCE* dosage by quantitative PCR

- *RHD/RHCE* dosage
- quantitative real time PCR
- Problem
  - *RHCE-D-CE* alleles, like *D--* primarily in Europeans (?)
- Resolution
  - hybrid *Rhesus box* by PCR-SSP

best would be both tests, but one test is better than none

- CE-labelled test kits
- offered as a service in Germany since 2001

- no test kit available
- offered as a service in Germany (not in Ulm)

- Blood 95(2000)3665
- Transfusion 45(2005)327&338

- Transfusion 46(2006)1343

Summary
Clinical applications in Germany

- Patients with weak D phenotypes
  - prevalent weak D should be transfused D pos.
- Maternal care
  - prenatal diagnostics
  - RhIg in pregnancy
- Blood donors
  - screen for DEL, weak D and D^+/D^- chimera among serologically D neg. donors
- Anti D and family planning
  - genotype father for *RHD* heterozygosity, if mother carries anti D
- Next step forward
  - mass scale genotyping, e.g. biochips

Supplementary material Editorials 2005

- Transfusion 45 (Mar 2005) 293

characterized and thus detected. Recently in Germany, PCR-based quality control of 15,045 serologically defined D– units revealed that 39 of them carried *RHD* alleles.[10]

- Transfusion 45 (Apr 2005) 466

many, *RHD* genotyping of D– first-time donors (among 500,000 donations/year) has been a routine procedure

- Transfusion 45 (Oct 2005) 1547

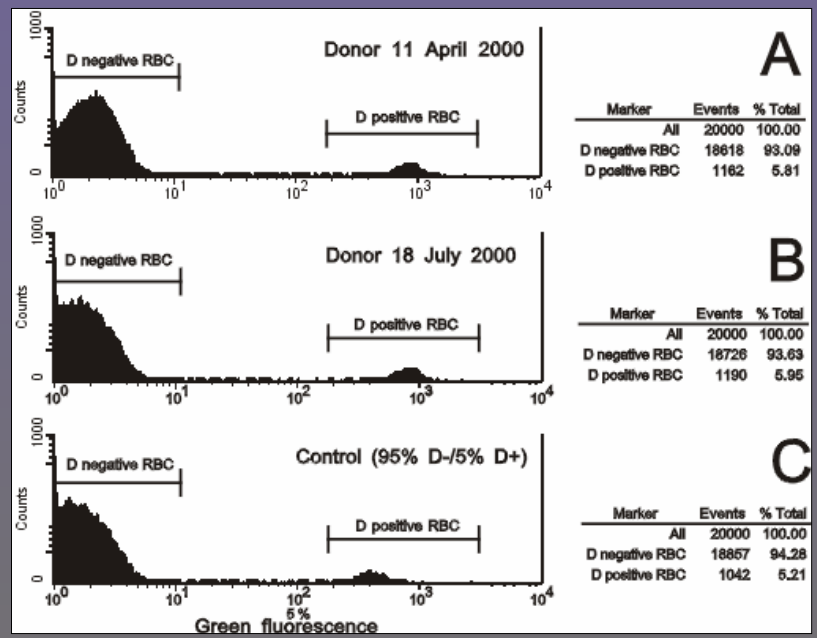
Since 2000, Flegel[5] has tested all his D– donors in Ulm, Germany, by a molecular method to ensure detection of all forms of *RHD*.

Anti D immunization by “D neg.“ blood donors

- weak D type 2
  - 1 immunization
    - Transfusion 40(2000)428
- D+/- chimera
  - > 2 immunizations
    - BMC Genet 2(2001)10
- weak D type 26
  - 1 immunization in pregnancy
    - Transfusion 45(2005)527
    - Editorial in Transfusion 45(2005)466
- DEL phenotype
  - *RHD*(IVS5-38del4)
  - 1 immunization
    - Transfusion 45(2005)520
- *RHD*(K409K)
  - most prevalent DEL type worldwide
  - 1 secondary immunization
    - Transfusion 45(2005)1581

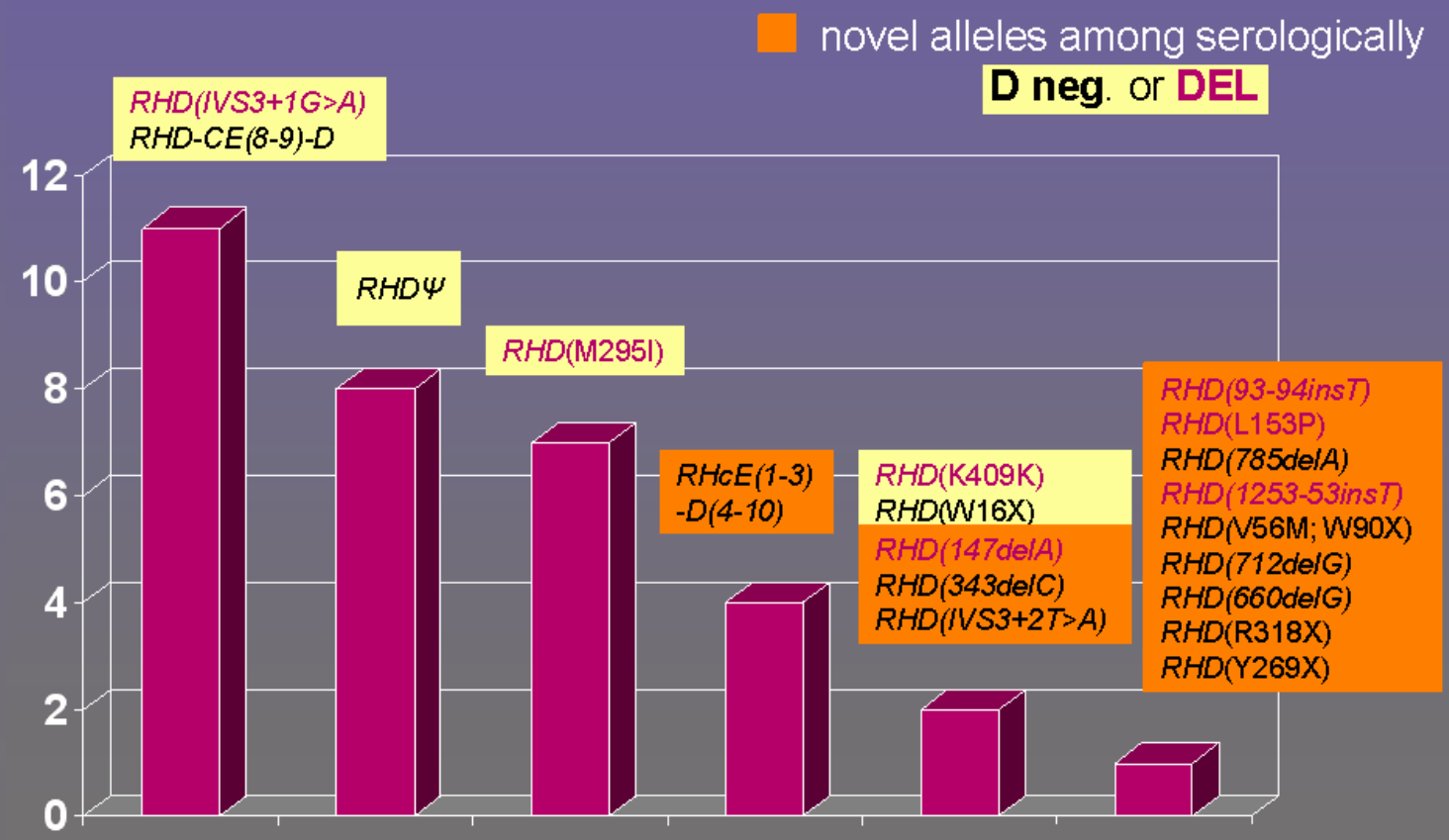
Superior sensitivity: mistyped by routine serology

- missed among 8,442 “D neg.“ donors
  - 3 partial D
  - 1 weak D type 2
  - 1 D+/- chimera
- D+/- chimera → total of 13 donations
  - caused anti-D in the latest 2 eligible transfusions
- it may be wise to revisit older serologic results

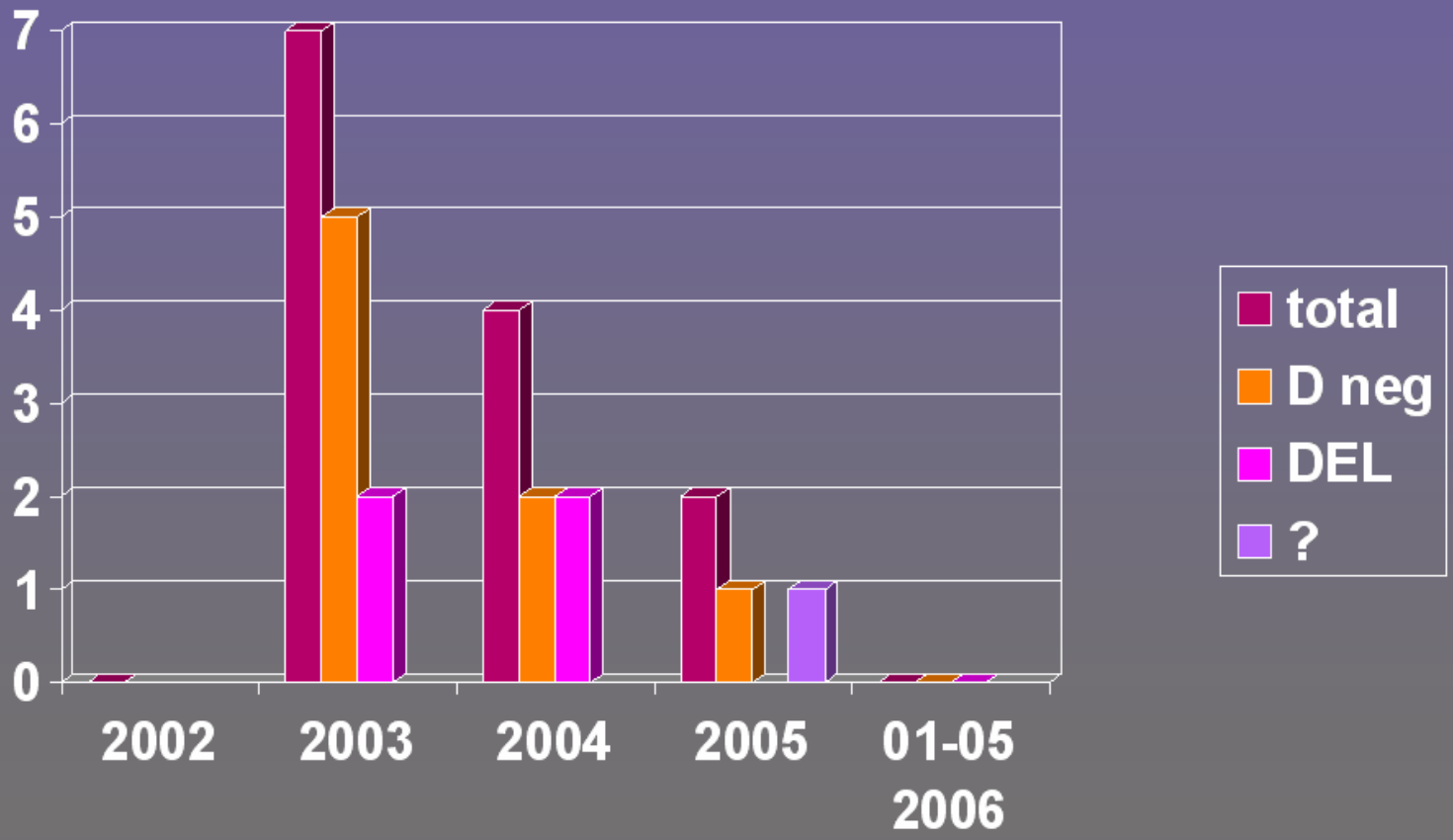


- BMC Genet 2(2001)10

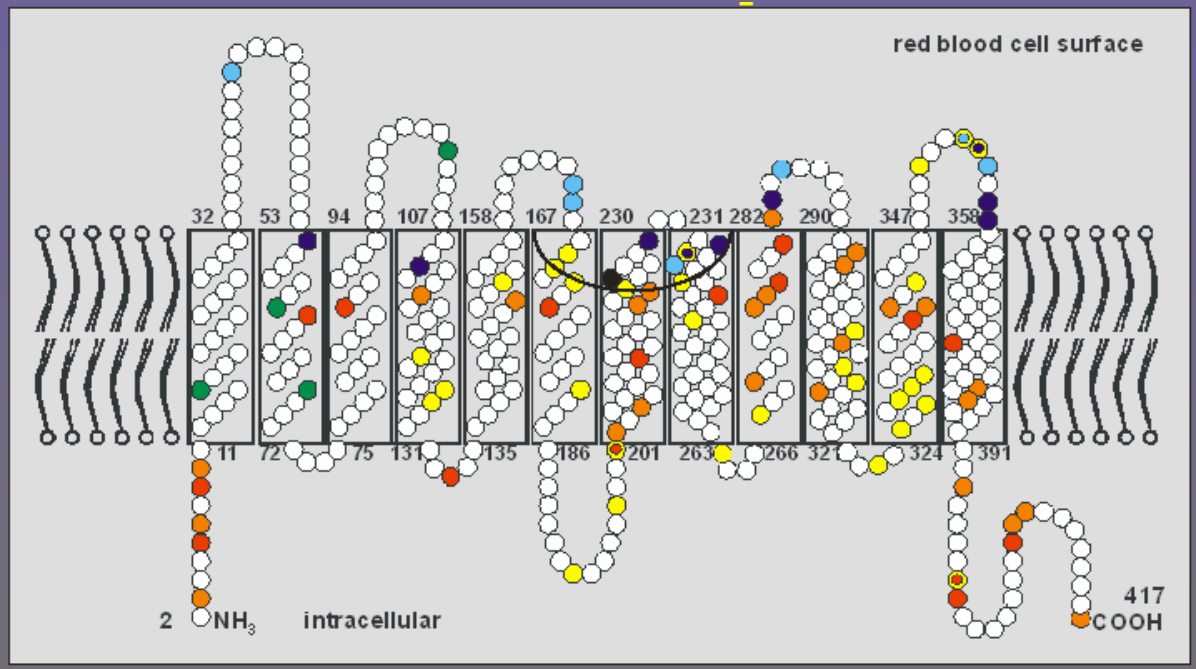
Frequency distribution



Novel alleles per year



RH blood group system RhD and RhCE proteins



Flegel WA: Curr Opin Hematol 2006 in press

- RhD vs. RhCE (yellow)
  - C/c (green)
  - E/e (black)
- weak D
  - (red/orange)
- partial D
  - (blue/light blue)

Type of D variant correlates with type of molecular variant

Type of variant	
D antigen	Allele (molecular type)
D category	*RHD-CE-D* hybrid alleles
partial D: European	exofacial single missense
partial D: African	exofacial dispersed missense
weak D	non exofacial single missense
DEL	e. g. splice site mutation
D negative	*RHD* deletion
	non functional rearranged
	non functional nonsense